PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : James W. Baumgartner et al.
Serial No. : 09/090,867
Filed : June 4, 1998
For : TESTIS-SPECIFIC RECEPTOR
Examiner : Lazar-Wesley, E.
Art Unit : 1646
Docket No.: 95-33D1
Date : July 21, 1999

Assistant Commissioner for Patents
Washington, D.C. 20231

Declaration Under 37 C.F.R. § 1.131

Sir:

We, James W. Baumgartner, Theresa M. Farrah, Donald C. Foster, Frank J. Grant, and Patrick J. O'Hara, do hereby declare as follows:

1. We are the inventors of the above-identified patent application.

2. All of the work described herein was performed in the United States of America by us or under our direction.

3. We have reviewed laboratory notes and other records, including the exhibits submitted herewith, and have determined that the invention recited in claims 1-32 of the above-identified patent application was reduced to practice before March 1, 1996 or was conceived before March 1, 1996 and was subsequently constructively reduced to practice with the filing of the patent application on March 13, 1996.

4. Attached hereto as Exhibit 1 is a copy of a computer printout of the DNA and deduced amino acid sequence of a clone designated "zcytor2." This printout is dated

prior to March 1, 1996. The sequences shown in Exhibit 1 correspond to those disclosed in the patent application in SEQ ID NO:1 and SEQ ID NO:2.

5. Attached hereto as Exhibit 2 is a copy of a portion of a memo written by one of us (Frank J. Grant) before March 1, 1996, which describes particular goals for the WSXWS receptor project, which project included the zcytor2 receptor. As stated in the memo, these goals included preparation of soluble forms (i.e., extracellular ligand-binding domains) of receptors. The memo also describes our intent to clone and express full-length, receptor-encoding cDNAs.

6. Attached hereto as Exhibit 3 is a copy of a page from the notebook of Cameron Brandt, a research associate working under our direction. This page, written before March 1, 1996, describes a plan to prepare polypeptide fusions comprising a soluble receptor and an immunoglubulin Fc polypeptide.

7. Attached hereto as Exhibit 4 is a copy of a slide prepared by one of us (Donald C. Foster) for an in-house seminar on the WSXWS receptor project. This slide was prepared before March 1, 1996. This slide illustrates a plan to express new receptor-encoding DNAs in cultured cells, whereby the cells would produce the encoded receptor.

8. On the basis of these Exhibits we conclude that the invention recited in claims 1-32 of the patent application was reduced to practice before March 1, 1996 or was conceived before March 1, 1996 and was subsequently constructively reduced to practice with the filing of the patent application on March 13, 1996.

We further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that the making of willfully false statements and the like is punishable by fine or imprisonment, or both, under

Section 1001 of Title 18 of the United States Code, and may jeopardize the validity of any patent issuing from this patent application.

James W. Baumgartner	Date
Theresa M. Farrah	Date
Donald C. Foster	9-10-99 Date
Frank J. Grant	9/23/99 Date
Patrick J. O'Hara	9/21/99 Date

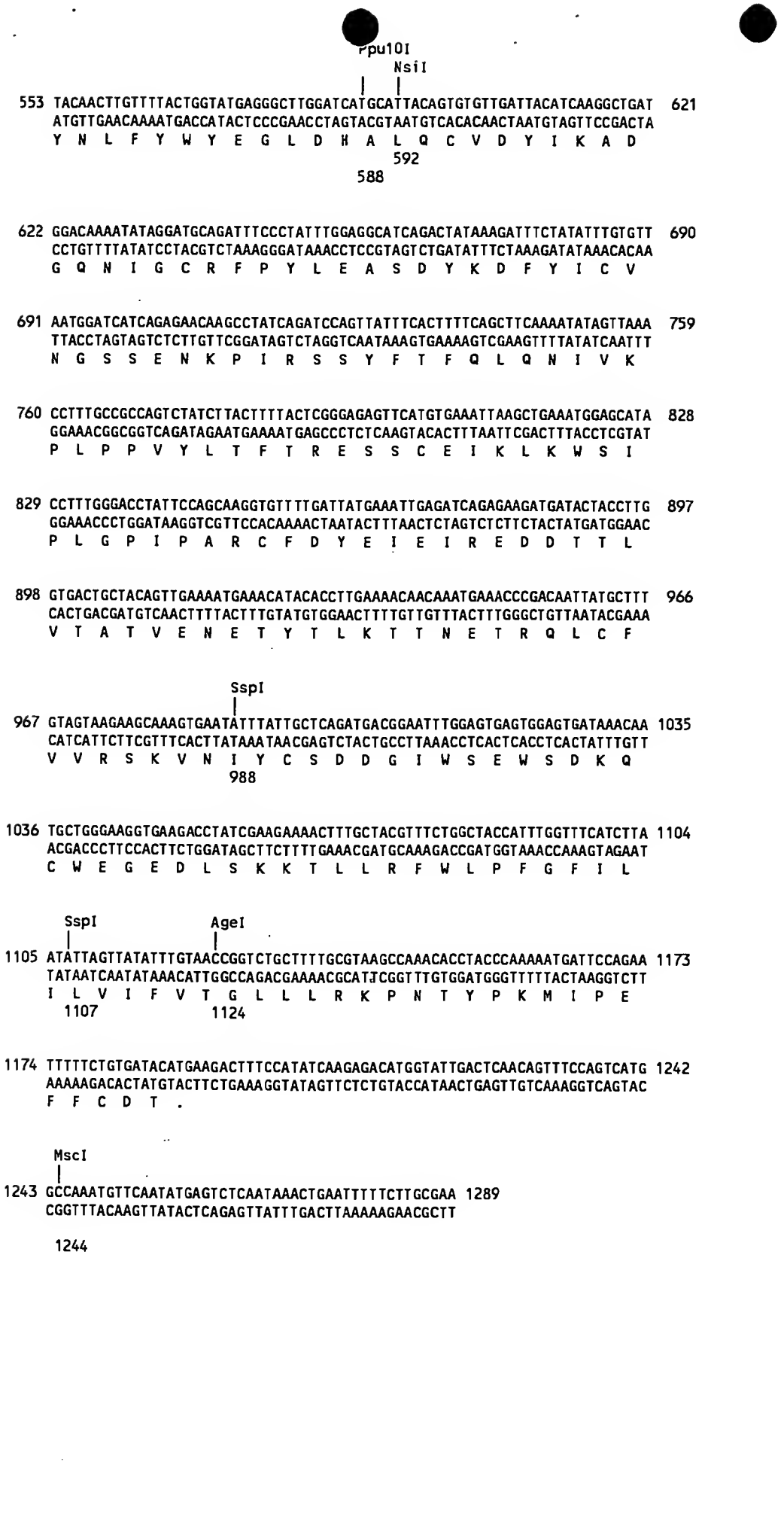
HZCYTOR02.SEQ -
Sequence of pcr products generated with 9800-9802,
nested pcr product 9941-AP2 (9801-AP1)
nested pcr product 9937-AP2 (9803-AP1)

Enzyme	Recognition	Cut Site		
AgeI	(A^CCGGT)	Def: 1124		
BamHI	(G^GATCC)	Def: 172		
DraI	(TTT^AAA)	Def: 36		
EcoRI	(G^AATTC)	Def: 450		
EcoRV	(GAT^ATC)	Def: 438		
HpaI	(GTT^AAC)	Def: 145		
MscI	(TGG^CCA)	Def: 1244		
MunI	(C^AATTG)	Def: 493		
NcoI	(C^CATGG)	Def: 377		
NsiI	(ATGCA^T)	Def: 592		
Ppu10I	(A^TGCAT)	Def: 588		
SmaI	(CCC^GGG)	Def: 11		
SspI	(AAT^ATT)	Def: 503	988	1107
XmaI	(C^CCGGG)	Def: 9		

HZCYTOR02.SEQ Linear LENGTH = 1289

```
            XmaI
              SmaI                      DraI
              | |                        |
   1 CCCCCCGCCCGGGAGAGAGGCAATATCAAGGTTTTAAATCTCGGAGAAATGGCTTTCGTTTGCTTGGCT   69
     GGGGGGCGGGCCCTCTCTCCGTTATAGTTCCAAAATTTAGAGCCTCTTTACCGAAAGCAAACGAACCGA
                                                    M  A  F  V  C  L  A
              11                        36
             9

  70 ATCGGATGCTTATATACCTTTCTGATAAGCACAACATTTGGCTGTACTTCATCTTCAGACACCGAGATA  138
     TAGCCTACGAATATATGGAAAGACTATTCGTGTTGTAAACCGACATGAAGTAGAAGTCTGTGGCTCTAT
     I  G  C  L  Y  T  F  L  I  S  T  T  F  G  C  T  S  S  S  D  T  E  I

           HpaI                      BamHI
           |                          |
 139 AAAGTTAACCCTCCTCAGGATTTTGAGATAGTGGATCCCGGATACTTAGGTTATCTCTATTTGCAATGG  207
     TTTCAATTGGGAGGAGTCCTAAAACTCTATCACCTAGGGCCTATGAATCCAATAGAGATAAACGTTACC
     K  V  N  P  P  Q  D  F  E  I  V  D  P  G  Y  L  G  Y  L  Y  L  Q  W
           145                       172

 208 CAACCCCCACTGTCTCTGGATCATTTTAAGGAATGCACAGTGGAATATGAACTAAAATACCGAAACATT  276
     GTTGGGGGTGACAGAGACCTAGTAAAATTCCTTACGTGTCACCTTATACTTGATTTTATGGCTTTGTAA
     Q  P  P  L  S  L  D  H  F  K  E  C  T  V  E  Y  E  L  K  Y  R  N  I

 277 GGTAGTGAAACATGGAAGACCATCATTACTAAGAATCTACATTACAAAGATGGGTTTGATCTTAACAAG  345
     CCATCACTTTGTACCTTCTGGTAGTAATGATTCTTAGATGTAATGTTTCTACCCAAACTAGAATTGTTC
     G  S  E  T  W  K  T  I  I  T  K  N  L  H  Y  K  D  G  F  D  L  N  K

                                  NcoI
                                  |
 346 GGCATTGAAGCGAAGATACACACGCTTTTACCATGGCAATGCACAAATGGATCAGAAGTTCAAAGTTCC  414
     CCGTAACTTCGCTTCTATGTGTGCGAAAATGGTACCGTTACGTGTTTACCTAGTCTTCAAGTTTCAAGG
     G  I  E  A  K  I  H  T  L  L  P  W  Q  C  T  N  G  S  E  V  Q  S  S
                                  377

                           EcoRV       EcoRI
                           |           |
 415 TGGGCAGAAACTACTTATTGGATATCACCACAAGGAATTCCAGAAACTAAAGTTCAGGATATGGATTGC  483
     ACCCGTCTTTGATGAATAACCTATAGTGGTGTTCCTTAAGGTCTTTGATTTCAAGTCCTATACCTAACG
     W  A  E  T  T  Y  W  I  S  P  Q  G  I  P  E  T  K  V  Q  D  M  D  C
                           438         450

           MunI      SspI
           |         |
 484 GTATATTACAATTGGCAATATTTACTCTGTTCTTGGAAACCTGGCATAGGTGTACTTCTTGATACCAAT  552
     CATATAATGTTAACCGTTATAAATGAGACAAGAACCTTTGGACCGTATCCACATGAAGAACTATGGTTA
     V  Y  Y  N  W  Q  Y  L  L  C  S  W  K  P  G  I  G  V  L  L  D  T  N
           493       503
```

EXHIBIT 1


Fpu10I
NsiI
553 TACAACTTGTTTTACTGGTATGAGGGCTTGGATCATGCATTACAGTGTGTTGATTACATCAAGGCTGAT 621
ATGTTGAACAAAATGACCATACTCCCGAACCTAGTACGTAATGTCACACAACTAATGTAGTTCCGACTA
Y N L F Y W Y E G L D H A L Q C V D Y I K A D
592
588
622 GGACAAAATATAGGATGCAGATTTCCCTATTTGGAGGCATCAGACTATAAAGATTTCTATATTTGTGTT 690
CCTGTTTTATATCCTACGTCTAAAGGGATAAACCTCCGTAGTCTGATATTTCTAAAGATATAAACACAA
G Q N I G C R F P Y L E A S D Y K D F Y I C V
691 AATGGATCATCAGAGAACAAGCCTATCAGATCCAGTTATTTCACTTTTCAGCTTCAAAATATAGTTAAA 759
TTACCTAGTAGTCTCTTGTTCGGATAGTCTAGGTCAATAAAGTGAAAAGTCGAAGTTTTATATCAATTT
N G S S E N K P I R S S Y F T F Q L Q N I V K
760 CCTTTGCCGCCAGTCTATCTTACTTTTACTCGGGAGAGTTCATGTGAAATTAAGCTGAAATGGAGCATA 828
GGAAACGGCGGTCAGATAGAATGAAAATGAGCCCTCTCAAGTACACTTTAATTCGACTTTACCTCGTAT
P L P P V Y L T F T R E S S C E I K L K W S I
829 CCTTTGGGACCTATTCCAGCAAGGTGTTTTGATTATGAAATTGAGATCAGAGAAGATGATACTACCTTG 897
GGAAACCCTGGATAAGGTCGTTCCACAAAACTAATACTTTAACTCTAGTCTCTTCTACTATGATGGAAC
P L G P I P A R C F D Y E I E I R E D D T T L
898 GTGACTGCTACAGTTGAAAATGAAACATACACCTTGAAAACAACAAATGAAACCCGACAATTATGCTTT 966
CACTGACGATGTCAACTTTTACTTTGTATGTGGAACTTTTGTTGTTTACTTTGGGCTGTTAATACGAAA
V T A T V E N E T Y T L K T T N E T R Q L C F
SspI
967 GTAGTAAGAAGCAAAGTGAATATTTATTGCTCAGATGACGGAATTTGGAGTGAGTGGAGTGATAAACAA 1035
CATCATTCTTCGTTTCACTTATAAATAACGAGTCTACTGCCTTAAACCTCACTCACCTCACTATTTGTT
V V R S K V N I Y C S D D G I W S E W S D K Q
988
1036 TGCTGGGAAGGTGAAGACCTATCGAAGAAAACTTTGCTACGTTTCTGGCTACCATTTGGTTTCATCTTA 1104
ACGACCCTTCCACTTCTGGATAGCTTCTTTTGAAACGATGCAAAGACCGATGGTAAACCAAAGTAGAAT
C W E G E D L S K K T L L R F W L P F G F I L
SspI
AgeI
1105 ATATTAGTTATATTTGTAACCGGTCTGCTTTTGCGTAAGCCAAACACCTACCCAAAAATGATTCCAGAA 1173
TATAATCAATATAAACATTGGCCAGACGAAAACGCATTCGGTTTGTGGATGGGTTTTTACTAAGGTCTT
I L V I F V T G L L L R K P N T Y P K M I P E
1107
1124
1174 TTTTTCTGTGATACATGAAGACTTTCCATATCAAGAGACATGGTATTGACTCAACAGTTTCCAGTCATG 1242
AAAAAGACACTATGTACTTCTGAAAGGTATAGTTCTCTGTACCATAACTGAGTTGTCAAAGGTCAGTAC
F F C D T .
MscI
1243 GCCAAATGTTCAATATGAGTCTCAATAAACTGAATTTTTCTTGCGAA 1289
CGGTTTACAAGTTATACTCAGAGTTATTTGACTTAAAAAGAACGCTT
1244

DRAFT

[redacted]

[redacted] DEPT.

Outline of things to consider for patent application of novel type I cytokine receptors

We have identified partial cDNA sequences for three new members of the type I cytokine receptor family. These receptors are characterized by a conserved cysteine pattern and an amino acid motif containing WSXWS. Members of this family include the receptors for TPO, EPO, Growth Hormone, Prolactin, IL-4, IL-7, IL-9, IL-2, IL-5, IL-3, GM-CSF, IL-6, CNTF, G-CSF and Leukemia inhibitory factor.

The main utility for these sequences would be to facilitate the cloning of the unknown ligands for the receptors. The receptors themselves (ie. soluble forms) might be potential therapeutics as well.

There are at least three ways the receptor sequence can be utilized to clone the ligands:

a). Make receptor dependent cell lines (as was done in the [redacted] project) for use in an expression cloning project.

b). Soluble forms of the receptor can be labeled and used as probes in an expression cloning system.

c). The receptor could be attached to various columns or other supports and used to purify the ligand.

Patentable entities: (???????)

a). The EST (expressed sequence tag) that allowed us to identify the partial sequence as novel member of the family.

i). Allows us to clone the full length cDNA.

b). The full length receptor encoding cDNA.

c). Homologues of the cDNAs. It may be that murine versions of these receptors are necessary for ligand dependent cell line cloning.

d). The ligands for the receptors.

e). AIDS therapies.

WHAT WE GOT:

[redacted]

a).

i).

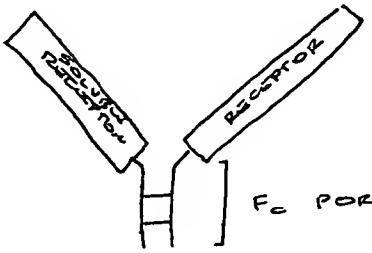
ii).

c).

EXHIBIT 2

[redacted]: CONSTRUCTION PLAN IgG γ_1 VECTOR

PURPOSE: WILL BUILD A VECTOR FOR EXPRESSION OF SOLUBLE RECEPTORS FUSED TO IgG γ_1 HEAVY CHAIN. THIS EXPRESSION SYSTEM ALLOWS AN EASY WAY TO PURIFY SOLUBLE RECEPTOR OVER A PROTEIN A COLUMN + THEN PROVIDES A HANDLE FOR USING IN ASSAYS AFTER LIGAND.



F_c PORTION OF IgG γ_1

- IgG PORTION OF FUSION INCLUDES HINGE REGIONS CH_2 & CH_3
- FUSION IS CONSTRUCTED AS A MONOMER BUT DIMERIZES VIA IT'S TWO CYSTEINE'S IN THE HINGE REGION

PLAN

ARG SER.

5': GLU - PRO - LYS - SER - CYS - ASP - LYS - THR - HIS - THR ...

↳ 1ST A.ACID OF HINGE REGION

↳ LYS CHANGED TO ARGININE TO ALLOW CONSTRUCTION OF Bgl II SITE (FANSLOW, ET AL. J. OF IMMUNOLOGY 149: 655-660

↳ CYS CHANGED TO SER JULY 15, 1992 TO ELIMINATE UNPAIRED CYS IN HINGE REGION. THIS CYS NORMALLY BIND LIGHT CHAIN, BUT LIGHT CHAIN IS NOT NECESSARY FOR CREATION OF A FUNCTIONAL FUSION (BENNETT, ET AL J. OF BIOL. CHEMISTRY 266 (34) 23060-23067 DEC 5, 1991)

3': WILL BE IDENTICAL TO NAT JL

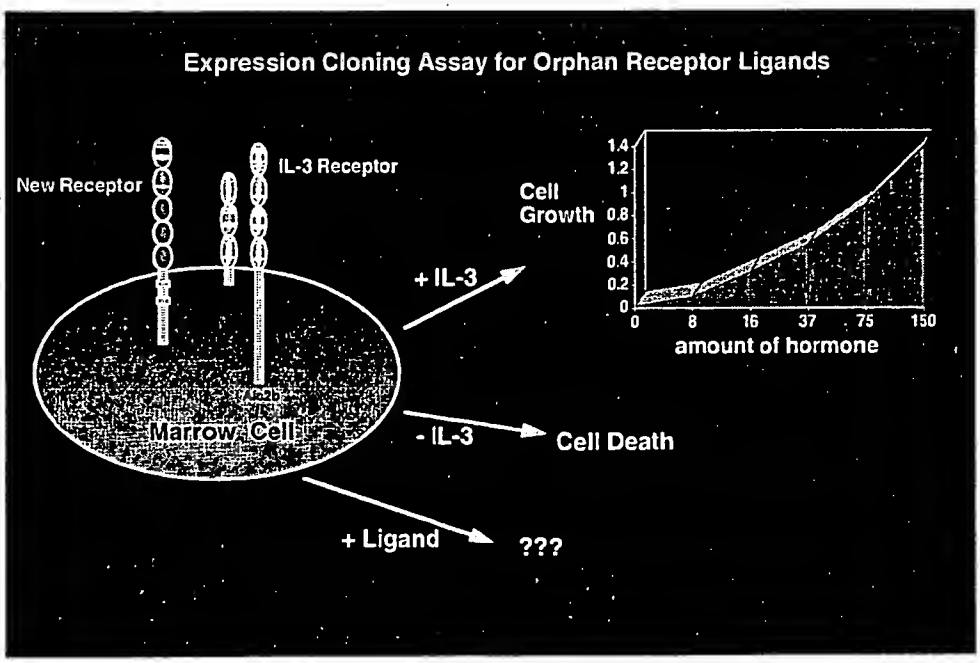

Expression Cloning Assay for Orphan Receptor Ligands
New Receptor
IL-3 Receptor
Marrow Cell
+ IL-3
Cell Growth
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
8
16
37
75
150
amount of hormone
- IL-3
Cell Death
+ Ligand
???

EXHIBIT 4